

IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method of quantitation of an amount of a protein which is glycated protein relative to the total amount of the protein (non-glycated and glycated) in a sample which comprises:

(a) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area, with an aliquot of biological sample sufficient to cover said measurement area;

(b) contacting said solid support matrix with an aliquot of a first buffer ~~sufficient to rinse off unbound protein~~ wherein said first buffer has a pH selected to allow both glycated and non-glycated forms of the protein to be bound bind to said solid support matrix and wherein said first buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix;

(c) quantitating amount of the protein bound to said measurement area using measurement of a selected property of said protein to give a first bound protein reading;

(d) contacting said solid support matrix with an aliquot of a second buffer ~~sufficient to rinse off unbound protein~~ wherein said second buffer has a pH selected to allow the glycated protein to ~~be bound bind~~ to said solid support matrix but where the non-glycated protein ~~is does~~ not substantially ~~bound bind~~ to said solid support matrix wherein said second buffer is applied in an amount sufficient to rinse off the non-glycated protein;

(e) quantitating amount of the protein bound to said measurement area using measurement of the property measured in step (c) to give a second bound protein reading; and

(f) calculating ~~percentage~~ relative amount of glycated protein using said first and second bound protein readings.

2. (Currently Amended) A method according to claim 1 wherein the property is measured is using an optical reading.

3. (Original) A method according to claim 2 wherein the optical reading is absorbance or reflectance at a specified wavelength.

4. (Original) A method according to claim 3 wherein the glyated protein is glyated hemoglobin.

5. (Original) A method according to claim 3 wherein the glyated protein is glyated albumin.

6. (Currently Amended) A method for quantitation of an amount of a glyated protein which is glyated relative to the total amount of the protein (non-glyated and glyated) in a biological sample which compromises:

(a) contacting a solid support matrix which comprises a negatively charged group and a hydroxyboryl compound and which has a measurement area with an aliquot of a biological sample sufficient to cover said measurement area;

(b) contacting said solid support matrix with an aliquot of a first buffer ~~sufficient to rinse off unbound protein~~, wherein said first buffer has a pH of about 5.0 to about 7.0 and wherein said first buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix at a pH of about 5.0 to about 7.0;

(c) quantitating amount of the protein bound to said measurement area using measurement of a selected property of the protein to give a first bound protein reading;

(d) contacting said solid support matrix with an aliquot of second buffer ~~sufficient to rinse off unbound protein~~, wherein said second buffer has a pH of about 8.0 to about 10.0 and wherein said second buffer is applied in an amount sufficient to rinse off protein which does not bind to the solid support matrix at a pH of about 8.0 to about 10.0;

(e) quantitating amount of the protein bound to said measurement area using

measurement of a selected property of the protein to give a second bound protein reading; and

(f) calculating percentage relative amount of the glycated protein in said sample using said first bound protein reading and said second bound protein reading.

7. (Currently Amended) A method according to claim 6 wherein said first and second bound protein readings measure the same selected property of the protein.

8. (Currently Amended) A method according to claim 7 wherein the selected property is measured is using an optical reading.

9. (Original) A method according to claim 8 wherein the optical reading is absorbance or reflectance at a specified wavelength.

10. (Original) A method according to claim 9 wherein the glycated protein is glycated hemoglobin.

11. (Currently Amended) A method according to claim 9 wherein the glycated protein is glycated ~~albumium~~ albumin.

12. (Currently Amended) A method of quantitation of an amount of glyated hemoglobin which is glyated relative to total amount of hemoglobin (non-glycated and glyated) in a biological sample which comprises;

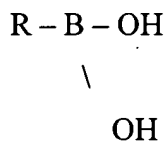
(a) adding said sample to a sample application site which is in communication with a solid support matrix which comprises a negatively charged group and a dihydroxyboryl compound and which has a measurement area;

(b) ~~making~~ adding an aliquot of a first buffer at said sample application site, wherein said first buffer has a pH between about 5.0 and about 7.0;

(c) making a first optical reading of said measurement area at a wavelength at which hemoglobin absorbs light;

- (d) adding an aliquot of a second buffer at said sample application site, wherein said second buffer has a pH between about 8.0 and about 10.0;
- (e) making a second optical reading of said measurement area at a wavelength at which hemoglobin absorbs light; and
- (f) calculating ~~the percentage relative amount~~ of glycated hemoglobin in said blood sample using said first and second optical readings.

13. (Original) A method according to claim 12 wherein said dihydroxyboryl compound has the formula



wherein R is selected from the group consisting of phenyl, substituted hydrogen, and alkyl of 1 to about 6 carbon atoms.

14. (Original) A method according to claim 13 wherein R is selected from the group consisting of phenyl, m-aminophenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

15. (Original) A method according to claim 14 wherein R is m-aminophenyl.

16. (Original) A method according to claim 13 wherein the negatively charged group is selected from the group consisting of carboxylate, sulfate, sulfonate, sulfinate and phosphate.

17. (Original) A method according to claim 16 wherein the negatively charged group is carboxylate.

18. (Original) A method according to claim 12 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylamide copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.

19. (Original) A method according to claim 18 wherein said solid support matrix is carboxy cellulose.

20. (Original) A method according to claim 12 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.

21. (Original) A method according to claim 12 wherein said second buffer is ammonium acetate or taurine.

22. (Withdrawn - Currently Amended) A method of quantitation of an amount of a non-hemoglobin glyeated protein which is glycated relative to total amount of the protein (non-glycated and glycated) in a biological sample wherein said protein is optionally labeled with a protein specific labeling agent which comprises:

(a) adding said sample to a sample application site which is in communication with a solid support matrix which comprises a negatively charged group and a dihydroxyboryl compound and which has a measurement area;

(b) adding an aliquot of a first buffer to said sample application site, wherein said first buffer has a pH between about 5.0 and about 7.0;

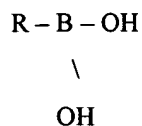
(c) making a first optical reading of said measurement area at a wavelength at which said non-hemoglobin protein or said labeling agent absorbs light;

(d) adding an aliquot of a second buffer to said sample application site wherein said second buffer has a pH between about 8.0 and about 10.0;

(e) making a second optical reading of said measurement area at a wavelength at which said non-hemoglobin protein or said labeling agent absorbs light; and

(f) calculating ~~the percentage relative amount~~ of glycated non-hemoglobin protein in said sample using said first and second optical readings.

23. (Withdrawn) A method according to claim 22 wherein said dihydroxyboryl compound has the formula:



wherein R is selected from the group consisting of phenyl, substituted phenyl, hydrogen, and alkyl of 1 to about 6 carbon atoms.

24. (Withdrawn) A method according to claim 23 wherein R is selected from the group consisting of phenyl, m-amino phenyl, hydrogen, ethyl, 1-propyl and 2-methyl-1-butyl.

25. (Withdrawn) A method according to claim 23 wherein R is m-aminophenyl.

26. (Withdrawn - Currently Amended) A method according to claim 21-22 wherein the negatively charged group is selected from the group consisting of carboxylate, sulfate, sulfonate, sulfinat and phosphate.

27. (Withdrawn) A method according to claim 26 wherein the negatively charged group is carboxylate.

28. (Withdrawn) A method according to claim 22 wherein said solid support matrix is selected from the group consisting of cellulose, nitrocellulose, cellulose acetate, polyacrylamide, agarose polyacrylamide copolymer, agarose, starch, nylon, nylon polyesters, dextran, cross-linked dextran, dextran acrylamide copolymer, cross-linked hydroxyethylmethacrylate substituted cross-linked polystyrenes, and polyvinylalcohol.

29. (Withdrawn) A method according to claim 28 wherein said solid support matrix is carboxy cellulose.

30. (Withdrawn) A method according to claim 22 wherein said first buffer is selected from the group consisting of MES, MOPS and HEPES.

31. (Withdrawn) A method according to claim 22 wherein said second buffer is ammonium acetate or taurine.

32. (Withdrawn) A method according to claim 22 wherein said sample is labeled with a protein specific labeling agent.

33. (Withdrawn) A method according to claim 22 wherein said sample is serum or plasma.

34. (Withdrawn) A method according to claim 33 wherein said non-hemoglobin glycosylated protein is glycosylated albumin.

Claims 35 to 40 (cancelled).